A Comparison of *Rhizobium* Strains for Effective Nodulation in Kenya Clover, *Trifolium semipilosum*[1]

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Trifolium semipilosum, Fres. (Kenya clover) shows promise of becoming an important cultivated pasture legume. It is apparently better able to withstand drought than *T. repens* (Britten 1962). Clovers are important as pasture plants because of their own high protein content and their ability to transfer nitrogen to associated pasture grasses. Since the ability to produce nitrogen depends on their being nodulated with an effective strain of *Rhizobium,* the success of a clover in a pasture is, therefore, directly related to the availability of an effective strain of *Rhizobium.* It is well known that many of the cultivated European species of *Trifolium* are successfully inoculated with a common inoculum. In one of the few papers on *Trifolium semipilosum,* originating outside of Africa, Hosaka and Matsuura (1958) state that the inoculum generally used for white clover may also be used for Kenya clover. Norris (1959) on the other hand has shown that the African species of *Trifolium,* including *T. semipilosum,* are not effectively nodulated with *Rhizobium* derived from European species of *Trifolium.*

The objective of this experiment was to compare the effectiveness of a *Rhizobium* inoculum effective on certain European species of *Trifolium* with a strain originally isolated from *T. semipilosum.*

Materials and Methods

Seed of *Trifolium sempilosum* was obtained from A. V. Bogdan, Grasslands Research Station, Kitale, Kenya. Inoculum for *T. semipilosum* was obtained from D. O. Norris, Cunningham Laboratory, C.S.I.R.O., Brisbane, Australia. This inoculum, designated CB782, was then increased and prepared for seed inoculation by the courtesy of Joe C. Burton of the Nitragin Company of Milwaukee, Wisconsin. Inoculum of the European *Trifolium* (Culture "B") group was also obtained from the Nitragin Company. Grateful acknowledgement is made to the above-mentioned workers in generously providing materials.

The first experiment was designed to determine the effectiveness of the *Rhizobium* cultures on established plants. Because of the nature of the experiment, it could not be done under aseptic conditions. Since CB782 had never been used experimentally or otherwise in Hawaii prior to thiis, there would be no opportunity for accidental inoculation of the plants by this culture. The plants used had been grown for another purpose and at the beginning of the experiment were about three months old. They were grown in non-sterile soil, and because inoculum for white clover had been used in the nursery, it is assumed that strains of *Rhizobium* effective on *T. repens* had opportunity to infect these plants. When the experiment began, the seedlings were in a poor nutritional status, being stunted in growth because of leaching. Foliage was pale greenish yellow, with some of the leaves displaying a bright red color. Plants were removed from the original pots and most of the adhering soil was washed away. Many small white nodules were found on the roots of most plants. The plants were placed in 12-ounce containers which were filled with vermiculite.

The plants were divided into three groups, conscious effort being made to ensure an equal distribution of different sizes. One group was then inoculated with an inoculum suitable for *T. repens,* culture "B", the second with CB782 and the third left uninoculated.

The summer environment of Honolulu is not favorable for growth of *T. semipilosum,* so the experiment was further divided into three environmental subtreatments. One group was placed on the open bench, receiving full sunlight, the second was placed on a bench which was partially covered so that it received no direct sunlight from about 10:45 A.M. to 1:15 P.M. A third group was placed in controlled environment cabinets (Britten and Kinch 1960) with temperatures set at 72±1 °F and 52±1 °F for day and night respectively. Photoperiod was set at 14 hours. Plants inoculated with CB782 were placed in a different cabinet from those inoculated with the *T. repens* inoculum and the controls. There were 15 plants in each of the ultimate treatment groups or 135 plants altogether. Plants were given nutrient solution three times a week and on other days given tap water. The nutrient solution was a slightly modified Hoagland's containing no nitrogen.

Notes were made on appearance and survival of the plants, nodulation of the roots and dry weights of tops 57 days after treatment.

The second experiment was begun under aseptic conditions and was designed to determine effectiveness of the *Rhizobium* strains on young seedings. Seed of *T. semipilosum* was scarified in concentrated sulphuric acid for 30 minutes, washed, dried and surface sterilized with a 1% solution of sodium hypochlorite. Seeds were rinsed several times in sterile water and then placed, with sterile forceps, in a mixture of vermiculite and pearlite which had been steam sterilized in 12 ounce containers.

Four inoculation treatment groups were established, one receiving type "B" inoculum, one CB782 and two groups no inoculum. Both inoculum groups and one uninoculated group received sterile nutrient solution lacking nitrogen; the remaining uninoculated group received sterile complete nutrient solution. Plants were watered on alternate days with distilled water. The experiment was designed to have four plants per pot with ten pots per treatment. The experiment was terminated at 51 days. Notes were taken on appearance, dry weight of tops and nodulation of roots. Nitrogen analysis of tops was done by the Kjeldahl method.

Two environmental treatment subgroups were set up, one on open benches, the other in the controlled environment cabinet with temperature and photoperiod set as before.

Results

The results of the experiment on

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[1] *Published with the approval of the Director of the Hawaii Agricultural Experiment Station as Technical* Paper No. 682.

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Table 1. Survival, dry weight and nitrogen content of established *Trifolium semipilosum* plants growing under three inoculation treatments and three environmental sub-treatments.

	Survival			Dry Weight Tops Per Plant			Nitrogen Per Plant			Ratio N CB782/"B"
Sub-treatment	No Inocul.	"B"	CB782	No Inocul.	"B"	CB782	No Inocul.	"B"	CB782	
	percent			grams			milligrams			
Partial shade	0	7	7	—	0.07	0.35	—	1.0	8.0	8.2
Open bench	13	33	40	0.09	0.13	0.55	1.3	1.5	9.5	6.3
Cabinet	73	80	80	0.21	0.16	0.29	2.3	1.6	7.9	4.9

Table 2. Dry weights, and nitrogen content of *Trifolium semipilosum* seedlings growing under four inoculation and nutrition treatments and two environmental sub-treatments.

	Dry Weight Tops Per Plant				Nitrogen Content Per Plant				
	Complete nutrient	Nutrient, -N			Complete nutrient	Nutrient, -N			Ratio N CB782/"B"
Sub-treatment	No Inocul.	No Inocul.	"B" Inocul.	CB782 Inocul.	No Inocul.	No Inocul.	"B" Inocul.	CB782 Inocul.	
	grams				milligrams				
Open bench	0.574	0.006	0.006	0.137	18.37	—	0.16	2.53	15.4
Cabinet	0.252	0.002	0.004	0.039	9.03	0.03	0.06	0.56	9.3

the established plants are summarized in Table 1. The environmental sub-treatments showed the greatest differential in mortality and growth. Only two plants survived under partial shade. Plants on the open bench also suffered high casualty with the group inoculated with CB782 having the highest survival rate.

The cabinet treatment sub-group showed the highest survival, with approximately equal numbers of each inoculation treatment group surviving.

The plants receiving CB782 made a slow but definite recovery from the poor condition at which they started. This was shown by the leaves taking on a darker green color and the disappearance of the red pigmentation. The other two groups maintained their pale greenish yellow and red coloration.

Growth of the plants, as indicated by dry weight of tops, was greater for those receiving CB782 inoculum than for the other two groups. Amount of nitrogen per plant was four to eight times greater for this group compared to the other two. Dry weight and nitrogen content of plants receiving culture "B" was less than those not receiving any deliberate inoculum.

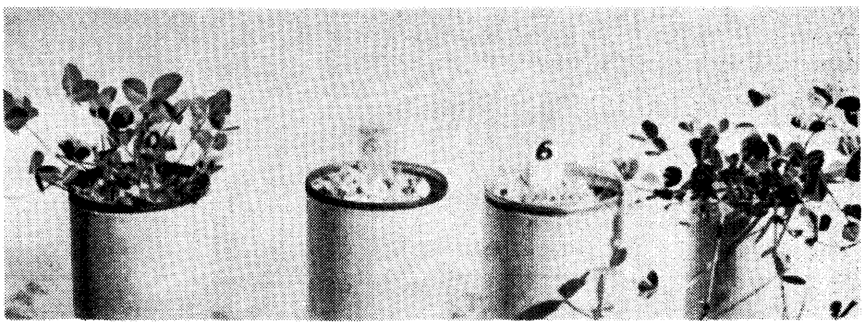

FIGURE 1. Plants of *Trifolium semipilosum* under different treatments with inoculum and nutrient solution. Plants from left to right are: (1) inoculum-CB782, nutrient-complete minus nitrogen; (2) inoculum-"B" suitable for European clovers such as *T. repens*, nutrient-complete minus nitrogen; (3) (negative control) inoculum-none, nutrient-complete minus nitrogen; (4) (positive control) inoculum-none, nutrient-complete including nitrogen. The plants in the two center containers were hardly visible both because of small size and pale color.

The results of two environmental sub-treatments in the second experiment, open bench and cabinet, parallelled each other for all treatments except that there was not enough plant material in the no-inoculum no-nitrogen treatment group for analyses even by micro-Kjeldahl. The second experiment had a treatment group not included in the first, namely, uninoculated and receiving complete nutrient solution. Growth of plants in this group was greatest of all as shown in Figure 1 and in Table 2. Growth of plants receiving treatment with CB782 was slow at the beginning, but improved as time went on. They were a healthy green color. Plants in both the uninoculated control not receiving nitrogen and the plants receiving the "B" clover inoculum failed to make satisfactory growth. They were stunted and pale yellow in appearance and at the time of termination of the experiment were barely maintaining themselves. Nitrogen content of the plants was highest in the plants receiving complete nutrient, less in those inoculated with CB782 and very much less in the uninoculated no nitrogen and the clover "B" inoculation group.

Examination of the roots of the plants showed two specific types of nodules. The type found on plants inoculated with "B" culture was white, spherical, numerous, and less than a millimeter in diameter. The nodules found on the plants of the first experiment grown in soil were similar in appearance. Nodules of the second type, found on plants inoculated with CB782, were large convoluted and at times reached a size of about 2 x 2 x 4 mm. When cut open they were greenish-pink in color. Nodules of both types were found on the same plants in the first experiment in the group inoculated with CB782. The small ineffective nodules were present at the beginning of the experiment. Some nodules of the CB782 type were also found on the distal parts of some plants inoculated with culture "B". These occurred on roots which had grown out of the bottom of the pots. Infection had occurred late and had not had an opportunity to affect growth of the plants significantly as was obvious from the appearance of the plants.

Discussion

The results of both sets of experiments clearly show that "B" inoculum used for inoculation of *Trifolium repens* and similar clover can and does produce nodules on *Trifolium semipilosum*, but these nodules are small and completely ineffective in fixing nitrogen with this species of clover. Plants treated with this inoculum failed to make satisfactory growth in the absence of added nitrogen. Plants grown in soil in the first experiment showed severe symptoms of nitrogen deficiency even though well nodulated. Analysis of plants treated with this inoculum were not greatly different from the controls which were not inoculated at all. They were consistently lower in nitrogen than the plants inoculated with CB782, as indicated in the last column of Tables 1 and 2. The ratio figures in Table 1 do not reflect the true nitrogen fixation picture for the two groups, as these plants had originally been grown in soil with added nitrogen. At the beginning of the experiment, the plants had a certain nitrogen reserve, both groups being presumably equal. That the plants treated with "B" inoculum failed to maintain this equality is shown by the ratio of the nitrogen level of the plants treated with CB782 compared to those treated with "B" *Rhizobium*. These ratios indicate a mean of 6.5 times more nitrogen for the CB782 treatment.

The results of the second experiment also indicate the inability of *Rhizobium* "B" culture to be effective on *Trifolium semipilosum*. This is shown by lack of growth and low nitrogen content of the plants. Plants inoculated with CB782 showed a healthy green color.

Plants receiving full nutrient solution grew more rapidly than those receiving the CB782 treatment. This difference would be expected when it is realized that plants growing with full nutrient received adequate amounts of nitrogen from the beginning, while the uninoculated plants received no nitrogen except that carried in the seed, until nodulated by the symbiotic organism. Under field conditions, it would be expected that some nitrogen would be available to the seedlings at time of germination. The second experiment in which plants were grown in vermiculite with no added nitrogen except in one control group was, therefore, a more severe test than would normally be encountered in the field.

The experiments also indicate differences in growth and survival of Kenya clover plants when grown under different environments. The environmental sub-treatments were given only to ensure that at least one set of experiments would give the required information on nodulation effects. It is fortunate that the experiment was planned in this manner, because the plants grown on benches during the summer (Experiment 1) did not give as much information as those grown in the cabinet. The experiment was not designed to test the optimum environment for *T. semipilosum* so that this aspect of environment subtreatments needs no further discussion.

It can be concluded that *Trifolium semipilosum* is not effectively nodulated with strains of *Rhizobium* commonly effective on European clover species. Strain CB782 is available for effective inoculation of this species.

LITERATURE CITED

BRITTEN, E. J. 1962. Evaluation for pasture purposes of some African clovers in a plant introduction program. Jour. Range Mangt. 15: 329-333.

BRITTEN, E. J. AND D. M. KINCH. 1960. A low-cost controlled environment cabinet with diurnal temperature fluctuation. Ecology 41: 785-790.

HOSAKA, E. Y. AND M. MATSUURA. 1958. Kenya white clover in Hawaii. Univ. of Hawaii Agr. Ext. Serv. Circ. 369. 6 pp.

NORRIS, D. O. 1959. *Rhizobium* affinities of African species of *Trifolium*. Empire Jour. Exptl. Agr. 27: 87-97.

Grazing Susceptibility Numbers of Grasses and Forbs from the Grazing Grounds of Varanasi, India.

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Considerable work has been done on the phytosociological aspects of grassland communities in relation to grazing (Shepperd, 1919, 1939; Humphrey and Mehrhoff, 1958; Weaver and Hansen, 1941; Drew, 1947; Kucera, 1956 and Launchbaugh, 1955). Ellison, (1960) while studying the influence of grazing on plant succession of rangelands tried to give successional trends by the major plant types of the United States, beginning with the true prairie of the Great Plain and moving Westward. He gave more stress on changes resulting from different intensities of grazing.

Hence the vegetation is typified by the general features of the characteristic flora and abundance on the grounds. It readily responds to changes in the habitat; and if it is repeatedly mowed, scraped, tram-

[1] *I am deeply indebted to Dr. R. Misra, F.N.I., Professor and Head of the Botany Department, Banaras Hindu University, Varanasi, for valuable guidance, for kindly going through the manuscript, and for helpful suggestions. I am also thankful to the Government of India for financial assistance in the form of Research Scholarship.*